A New Perspective Of Hydrolysable Tannin As A Treatment For Pigmentation Problem

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Abstract

Hyperpigmentation is a harmless but give significant distress to the affected individual that affecting quality of life is a common dermatological condition in which the color of the skin generally becomes darker. This discoloration of the skin can be a result of a various internal and external factors including hormonal changes, inflammation, injury, certain medication and ultraviolet (UV) exposure. The biological process involving the production of the skin pigment called melanin produced by melanocytes in various skin layers of the skin. Melanogenesis, the process of melanocytes synthesis melanin can be altered, producing pigmentary skin disorders by the changes in melanocyte production or melanin distribution. Tannin are phenolic compounds found in varies plants and have been used throughout history for their pharmacological properties as part of plants and herbs in traditional medicine. The study of tannins, especially hydrolysable tannin as a part of tannin group shows the effectiveness of their apparent use on skin pigmentation problem. This scientific approach about hydrolysable tannin as a part of tannin may pose an attractive alternative treatment for hyperpigmentation problem. Through this review, it is hoped that we will gain a better understanding of how hydrolyzed tannins are a promising alternative to treat hyperpigmentation.

Keywords: tannins, hydrolysable tannin, phenolic compound, hyperpigmentation, melasma, photoaging, skin, tyrosinase, anti-tyrosinase

1. Introduction

Skin is the most vulnerable tissue due to its exposure to the external environment. Acute exposure of Ultraviolet Radiation (UVR) make an inflammatory response and erythema, whereas chronic exposure linked to carcinogenesis and photoaging of the skin.[1] Hyperpigmentation disorders are treated with a wide range of topical skin lightening agents, chemical peels, laser therapy or either combination.[2]

Skin pigmentation is a complex process involve the synthesis of melanin as the principal to determine skin color.[3] Melanin is synthesized and accumulated within the melanosome, organelle inside the melanocyte. Melanocytes are

melanin pigment-producing cells that localize to multiple anatomic sites, most prominently the epidermis.[4] Melanin

provide protection by limiting UVR absorption by approximately 50 – 75% and scavenging ROS.[3] The two melanin pigment divided into eumelanin which has black or brown hue and pheomelanin which has yellow or red hue. After melanin is synthesized, pigment containing melanosomes are transported to keratinocytes, internalized and trafficked to perinuclear locations where they can absorb UV light and protect keratinocyte nucleus from Ultraviolet (UV)- associated radiation damage.[4]

Pigmentation disorders of the skin exist as hypopigmentation and hyperpigmentation in a variety of benign and malignant contexts.[4] Hyperpigmentation of the skin usually harmless, such as melasma, solar lentigines and freckles give significant cosmetic nuisance and distress to the affected individual. Melasma presents with dark patches that distributed on the face or neck as hyperpigmented macules over the skin. Freckles are flat, small patch that predominantly present on sun-exposed areas of the skin. Lentigines marked by presence of a small brown patch on sun-exposed areas. What differ freckles to lentigines are in freckles, the numbers of melanocytes remains the same but there is an increased amount in melanin, whereas, lentigines result from an increased number of melanocytes.[2]

Photo oxidation of pre-existing melanin will result in immediate and persistent pigment darkening. UV rays initiate photo oxidative reactions and activate protein kinase C and Reactive Oxygen Species (ROS) that react with protein, lipids and DNA to produce cyclobutene pyrimidine dimers which are responsible for oedema, skin burn, cell apoptosis and erythema.[5] While melanin plays a crucial role in protecting skin against harmful effect of UVR, excessive production of melanin could be detrimental because melanin precursors and intermediate metabolites produced during melanogenesis in response to UVR. Whereas hyperpigmentation as defensive response of the skin, alteration in melanin synthesis implicated in skin damage.[6]

The basic mechanism of skin darkening starts with UV exposure and continuous stimulation of α-MSH, a peptide hormone which promotes melanocytes production. When α-MSH bound to specific active areas on melanocyte surfaces, they induces melanogenesis via multiple pathways. MITF, a key transcription for regulating the transcription of melanogenic enzymes, especially tyrosinase.[3] Tyrosinase, a copper-containing membrane, located in melanosome, catalyzes hydroxylation of L-tyrosine to L-DOPA, which is the first and rate-limiting step of melanogenesis in both eumelanin and pheomelanin.[6] This mechanism will protect the skin from the detrimental effects of UVR, but an excessive production is responsible for skin hyperpigmentation.[3]

2. Melanogenesis Pathway

Melanogenesis in melanocytes is a complex process involving tyrosinase-catalyzed oxidation of tyrosine that happen in basal epidermal layer.[6,7] Signal transduction pathways that mediate the regulation of melanogenesis involve the binding of agonists to MC1R that trigger events inside melanocytes through raising intracellular cyclin 3'-5'- cyclin adenosine monophosphate (cAMP) and activating the adenylate cylclase enzyme, protein kinase A (PKA) leading to phosphorylation of cAMP responsive binding element (CREB), which promote MITF activation, tyrosinase-related protein 1 (TRP-1) and tyrosinase-related protein 2 (TRP-2).[6] Mitogen activated protein kinases (MAPK) including p38 MAPK recently reported involved in MITF regulation. Activation of p38 MAPK increases

tyrosinase (TYR) and stimulating melanogenesis. Second messenger derived from ATP, cAMP, plays a role in intracellular signal transduction. PKA has direct effect on melanogenesis and its activation leads to MITF expression by CREB phosphorylation which increase melanin synthesis.[7]

In melanogenesis pathway, there are 3 main enzymes that are involved: TYR, TRP-1 and TRP-2.[2,7] Melanogenesis initiated by either hydroxylation of phenylalanine into L-tyrosine or directly by L-tyrosine, which is then hydroxylated to L-dihydroxyphenylalanine (L-DOPA). L-DOPA oxidized into L-DOPA-quinone (DQ). Both of these reactions are catalysed by tyrosinase, a key-rate limiting enzyme in melanogenesis. After DQ is formed, melanogenesis pathway is divided into 2 parts, leading to the synthesis of black-brownish eumelanin and red-yellow pheomelanin. DOPA chrome is either spontaneously converted to 5,6-dihydroxyindole or enzymatically converted to 5,6-dihydroxyindole 2-carboxylic acid by TRP-2. The final product of polymerization of indole and quinones results in the formation of eumelanin. Pheomelanin synthesis is dependent on the cysteine presence, which react to DQ to form cysteinyl-DOPA and converted into quinoline then finally polymerizes to pheomelanin. Melanin plays key role in protecting the skin from harmful ultraviolet (UV) radiation.[2]

Underlying mechanisms in regulating pigmentation involve the direct suppression of tyrosinase activity and/or gene expression, direct scavenging ROS, promotion of Nrf2-regulated antioxidant defense and inhibition of signaling pathways. Skin exposure to the UVR can stimulate keratinocytes to secrete hormones including ACTH, ET-1, α-MSH that bind to MC1R, activating MITF and upregulating melanogenesis-related proteins. NRf2 activation can suppress the paracrine factors derived from keratinocytes that results in downregulation of signaling pathways involved in melanogenesis.[6]

3. Tannin Classification

Phenolic compounds have a wide range of different molecules, such as flavonoids, stilbenes, ligans, tannins and phenolic acids. Flavonoid are the most extensively studied of more than 8.000 phenolic compounds isolated from fruit and vegetables. An important substances yet neglected are tannins[8], they are high molecular weight (500 – 20.000 daltons).[8,9] Tannins are natural polyphenolic compounds which are found in a variety of plants.[8,10] Tannins are active secondary metabolites among polyphenols which play crucial role due to their promising medicinal properties.[10] Tannin are natural astringent materials[11] which are released from tannin vacuoles making complex with protein in oral cavity.[7]

The antioxidant of tannins works by stabilize free radicals and prevents photo-damage by partially inhibiting oxidation levels and photoaging.[11] Tannin are a heterogenous group of water soluble polyphenols[12], secondary metabolites in plants synthesized with as many as 20-hydroxyl groups.[9,12] Being phenolic compounds, tannins are highly hydrophilic molecules, chemically reactive and soluble in aqueous solvents as well as exhibiting a high

tendency to stably bond with proteins and carbohydrates.[9] Phenolic group of tannins binds very tightly with -NH groups of peptides and proteins. Tannins are ubiquitously present in barks, seeds or fruit peels of many vegetable species, but also in brown algae.[9]

Tannin classified under their functional units such: hydrolysable tannins (HT), condensed tannin (CT), phlorotannins (PT) and complex tannin (CoT).[8,9,11] Condensed tannin (CT) or proanthocyanidin, the most abundant plant derived polyphenols. Their structure are oligomers of flavan-3-ol (catechin monomers) and/or flavan-3,4-diol, usually linked by C-C (4-8 or 6-8) and occasionally by C-O-C bonds with a wide structural diversity. CT are not readi to hydrolyse, they decompose in acidic alcoholic and giving red pigments called phlobaphenes.[12] Hydrolysable tannin (HT) are readily hydrolyzed by acids, bases, hot water and some enzymes. The two group of HT exist as gallotannins (GT) and ellagitannins (ET)[9,13], also known as "tannic acid"[11], their name comes from either the gallic acid (GA) or ellagic acid (EA) unit obtained after hydrolysis.[8,9,13] Tannic acid structure consisting of a central glucose and ten galloyl group, water soluble polyphenol that is present in the bark and fruits of bananas, raisins, grapes, sorghum, spinach, coffee, persimmons, chocolate and tea had an effective superoxide, DPPH and ABTS radical scavenging activity, H_2O_2 scavenging activity. Fe^{3+} reducing power and metal chelation on ferrous ion activities[14]. Phlorotannins (PT) constituted upon molecules of phlorglucinol (PG, aromatic ring with 1,3,5 hydroxyl group) that polymerize with ease between C1-C3. Increasing complexity in their structure is correlated to a higher presence of PG subunits (3 – 7 subunits). On the other hand, complex tannin (CoT) are high molecular weight tannins resulting from the bonding of flavan-3-ols with either GT or ET via C-C bond[9]. A General perspective of tannin classification are presented.

HT esterified by either hexahydroxydiphenoyl acid (HHDP) or gallic acid (GA).[8] The core structure of GT either glucose or less commonly shikimic acid or quinic acid, which is esterified by up to five GA.[13] GT are polymers of galloyl coupled with polyol, catechin or triterpenoid units.[15] ET are product of oxidation of pentagalloylglucose[13] and mainly galloyl units organized through C-C coupling such as in hexahydroxydiphenol (HHDP), HHDP-esters or nonahydroxytriphenoyl (NHTP) esters subunits.[9] As these compounds are readily hydrolysable, castalin and vescalin, which are the nonahydroxydiphenoyl (NHDP) moiety esterified to an open-chain glucose, as well as EA, can be released after the hydrolysis of castalgin and vescalagin respectively.[13] After chemical or enzymatic hydrolysis, GT release gallic acid (GA), while ET release also HHDP, spontaneously converted to ellagic acid (EA).[15] HT and EA are well known as natural antioxidants [8] whose potency depends on their oxidation stage.[16] GT provide sugar and gallic acid on hydrolysis and ET which on hydrolysis do not yield sugar and gallic acid but also ellagic acid.[12] Plants containing HTs that are rich sources of GT and ET such as pomegranate (*Punica granatum L.*), berries (*Fragaria sp., Rubus spp.*), walnut (*Juglans regia L.*), sumac (*Rhus coriaria L.*), sweet chestnut *(Castanea sativa Mill.*) and oak (*Quercus spp.*).[15]

4. Biosynthesis Of Hydrolysable Tannins

Biosynthesis of all phenolics is based on the shikimate and acetate-malonate pathways, but the synthesis of tannins mostly by shikimate pathways.[10] It is known that the start of biosynthesis of HT begin by esterification of gallic acid

with glucose and the first intermediate is β-glucogallin (1-O-galloyl-β-d-glucose).[16,17] Without any donor, β- glucogallin acts as an acyl donor and acceptor of galloyl groups to produce further substituted glucose. Later,four consecutive galloylation reactions carried out: β-glucogallin → 1,6-digalloylglucose → 1,2,6-trigalloylglucose → 1,2,3,6-tetragalloylglucose → 1,2,3,4,6-pentagalloylglucose (PGG). PGG is a basic compound needed to synthesise GT and ET[13,16], where complex metabolites from GT can be formed through addition of 10 or more galloyl unit.[17] Galloyl groups may be further esterified by GA through depsidic bonds and up to thirteen groups.[13]

PGG was explicitly proposed as the immediate precursor of ET that should be produced by oxidative biaryl coupling of neighboring galloyl groups.[17] Biosynthesis derives from galloylation of PGG forms one or more meta depsidic digalloyl moieties, two galloyl groups joined by an ester bond. The depsidic bond formation is catalysed by galloyltransferases dependent on β-glucogallin. ET is formed by intra and intermolecular oxidation process of PGG, mediated by a laccase type polyphenol oxidase. The oxidation of PGG to tellimagrandiin II lead to the formation of the HHDP biaryl unit[16,17], which defines the hydrolysable tannins as ellagitannins. HHDP has a strong tendency to form ellagic acid through spontaneous lactonization from ellagitannins hydrolyzed.[16]

5. Hydrolysable Tannins As Anti-hyperpigmentation Therapy

The antioxidant activity of tannins is connected with the phenolic moieties in their structure which they can act as electron scavengers. Tannin activity and related polyphenols is highly dependent on the applied dose and the synergies established between acting molecules.[10] Many different compounds have been isolated from their anti tyrosinase activities being studied.[7] Hydrolysable tannins (HT) also have tyrosinase inhibition activity due to their protein binding capabilities. Ellagic and gallic acid has significant tyrosinase inhibition abilities.[18] Quercetin-3-O-β- d-glucopyranosid (QCGG) that inhibits melanogenesis and tyrosinase activity in B16F10 cells by suppressing expression of MITF, TRP-1, TRP-2, TYR via p38 MAPK and CREB pathway by reducing intracellular cAMP levels. Iso-quercetin and hyperin were found to be potent inhibitors of melanin production by suppressing tyrosinase expression in mouse B16 melanoma cells.[7] The possible mechanism was downregulation of MC1R receptor gene expression and involvement of cAMP/MITF/TYR pathway of melanogenesis regulation in these cells.[10] Epicatechin, epicatechin 3 gallate, epigallocatechin and gallocatechin as a part of HT, they work by inactivate ROS and helps to reduce DNA damage and incidence of erythema.[5]

In a study conduct by Jing (2022), tannic acid (TA) has been reported has protective effect against UVB radiation by decreasing UVB-induce ornithine decarboxylase activity and UVB-stimulated DNA synthesis, decrease IL-6 and IL-8 production by TNF-triggered keratinocytes. TA protective effect against UVB radiation by inhibiting the inflammatory response via mediation of IL-6/STAT3/CFB pathway.[1] Gallic acid (GA) alone shown to suppress melanogenesis by downregulating melanogenic regulatory genes in TYR, TRP-1 and dopachrome tautemerase expression at transcription and translation level. GA inhibits MITF expression by reducing cAMP-mediated PKA/CREB signaling cascade. Similar to chlorgenic acid acts as a substrate to melanin and its metabolic products, GA show its work by suppress melanogenesis in B16 melanoma cells by inhibiting TYR activity.[7] Studies conduct by

Liu (2021) shown that GA has excellent tyrosinase inhibition capability with an IC_{50} value 100-fold lower than kojic acid.[18]

6. Conclusion

Hydrolysable tannin (HT) as a part of phenolic compound is an important substances yet neglected. Major group of HT such as gallotannins (GT) and ellagitannins (ET) or known as tannic acid (TA) show potential effect in pigmentation problem by suppressing melanogenesis pathway. HT have their way to suppress melanogenesis pathway by their antityrosinase activities such as ellagic acid (EA) and gallic acid (GA) that shows excellent tyrosinase inhibition by downregulating melanogenic regulatory genes in TYR, TRP-1 and dopachrome tautemerase expression, inhibits MITF expression by reducing cAMP-mediated PKA/CREB signaling cascade and inhibiting TYR activity. Therefeore, it is necessary to continue investigations on the action mechanism which can help to use them in pharmaceutical formulations on daily use as a treatment for pigmentation problem.

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